

Report

A Locus for Autosomal Dominant “Pure” Hereditary Spastic Paraplegia Maps to Chromosome 19q13

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Summary

Genetic loci for autosomal dominant pure hereditary spastic paraplegia (ADPHSP) have been mapped to chromosomes 2p, 8q, 12q, 14q, and 15q. We undertook a genomewide linkage screen of a large family with ADPHSP, for which linkage at all previously identified ADPHSP loci was excluded. Analysis of markers on chromosome 19q gave a peak pairwise LOD score of 3.72 at D19S420, allowing assignment of a novel ADPHSP locus (which we have termed “SPG12”) to this region. Haplotype construction and analysis of recombination events narrowed the SPG12 locus to a 16.1-cM region between markers D19S868 and D19S902.

The hereditary spastic paraplegias (HSPs) are neurodegenerative disorders that are clinically characterized by progressive lower-limb spasticity. They are conventionally classified as pure when spastic paraplegia exists in isolation and as complicated when other major clinical features are present. Autosomal dominant, autosomal recessive, and X-linked-recessive inheritance patterns have been described for both pure and complicated forms of HSP (Harding 1984; Fink and Heiman-Patterson 1996; Kobayashi et al. 1996; Reid 1999).

There is locus heterogeneity within the autosomal dominant, autosomal recessive, and X-linked recessive

HSP inheritance groups. This is most striking for autosomal dominant pure HSP (ADPHSP), for which five loci have been mapped—on chromosomes 2p (SPG4 [MIM 182601]; Hazan et al. 1994; Hentati et al. 1994), 8q (SPG8 [MIM 603563] Hedera et al. 1999b; Reid et al. 1999b), 12q (SPG10 [MIM 604187] Reid et al. 1999a), 14q (SPG3 [MIM 182600] Hazan et al. 1993), and 15q (SPG6 [MIM 600363] Fink et al. 1995). The existence of at least one additional ADPHSP locus was strongly suggested by the exclusion of linkage at all five of these loci (with LOD scores <-2 throughout each candidate region) in a large Welsh family, family 28 (Reid et al. 1999a, 1999b, 1999c).

Family 28 (fig. 1) was ascertained as part of a previously reported U.K.-wide clinical and genetic study of ADPHSP (Reid et al. 1999c). After we obtained informed consent, all available affected and apparently unaffected family members were neurologically assessed by a single physician (E.R.), by use of a standard protocol. Diagnostic criteria for ADPHSP were based on those of Harding and have been described elsewhere (Harding 1981, 1984; Reid et al. 1999c). In brief, subjects were classified as being affected if they had lower-limb hyperreflexia in addition to at least one of the following: progressive spastic-gait abnormality, bilateral extensor-plantar reflex, and bilateral sustained (i.e., on at least five beats) ankle or knee clonus. Subjects were classified as being possibly affected if lower-limb hyperreflexia was present without other abnormal signs; and they were classified as being normal if they had an entirely normal neurological examination. Ethical approval for the study was granted by the Addenbrooke's Hospital ethical committee. In total, 18 family members were clinically assessed. In addition, DNA samples were available from an additional affected subject (II:4), who was a deceased obligate gene carrier, and from two spouses.

A genomewide screen, excluding the X chromosome (because male-to-male transmission was present), was

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Table 1**LOD Scores for Family 28, with Markers from the SPG12 Region**

MARKER	LOD SCORE AT $\theta =$						
	0	.05	.1	.2	.3	.4	.5
D19S414	−2.186	1.276	1.359	1.183	.823	.376	.000
D19S868	−3.722	−.458	−.129	.080	.085	.030	.000
D19S416	.997	.904	.810	.619	.423	.219	.000
D19S425	.273	.225	.180	.101	.043	.001	.000
D19S220	2.737	2.495	2.243	1.701	1.107	.473	.000
D19S223	2.992	2.712	2.421	1.802	1.135	.457	.000
D19S420	3.208	2.922	2.623	1.987	1.295	.565	.000
D19S918	3.201	2.920	2.625	2.000	1.318	.585	.000
D19S412	3.125	2.841	2.546	1.918	1.241	.536	.000
D19S545	1.911	1.817	1.679	1.318	.873	.385	.000
D19S902	−2.107	1.440	1.475	1.226	.832	.380	.000

* Values were calculated on the basis of data on affected patients and on two clinically normal subjects (ages 42 and 50 years, ≥ 20 years older than the oldest age at onset [i.e., 22 years] of disease in the family) who were coded as unaffected.

commenced on DNA samples from the family, by use of the ABI Linkage Mapping Set, version 2 (LMS2; PE Biosystems), as described elsewhere (Reid et al. 1999a). In addition to the LMS2 marker set, markers D19S868, D19S416, D19S425, D19S223, D19S918, D19S412, and D19S545 were used in genotyping. Primer sequences for these additional markers are available from either the Génethon microsatellite-linkage map (Dib et al. 1996) or the Center for Medical Genetics, Marshfield Medical Research Foundation Web site. PCR reaction and thermocycling conditions were identical to those used for the LMS2 marker set, except that the reactions for D19S918 and D19S412 contained 10% dimethyl sulfoxide and annealing temperatures were optimized for each marker.

Pairwise linkage analysis was done by use of the MLINK program of the FASTLINK, version 4.0P, package (Lathrop et al. 1985; Cottingham et al. 1993; Schäfer et al. 1994), accessed via the Genetic Linkage User Environment (GLUE) interface (UK Human Genome Mapping Project Resource Centre). For linkage calculations, the gene frequency for ADPHSP was assumed to be 10^{-4} , and male and female recombination rates were assumed to be equal. Maximum disease penetrance was assumed to be 99%, as suggested elsewhere (Hazan et al. 1994). Allele frequencies were assumed to be equal for all markers—except D19S425, D19S220, D19S223, D19S420, and D19S918, for which they were calculated by genotyping a panel of unrelated U.K. subjects.

Clinical information on family 28 has been presented elsewhere (Reid et al. 1999a, 1999b, 1999c, 1999d). All affected subjects had a progressive spastic-gait abnormality, in addition to lower-limb hyperreflexia, meeting the Hereditary Spastic Paraplegia Working Group diagnostic criteria for being “definitely affected” (Fink and

Heiman-Patterson 1996), as well as our own diagnostic criteria. The mean \pm SD age at onset of symptoms for affected subjects in family 28 was 6.9 ± 6.2 (range 5–22) years. One subject (III:3) who had a nonspastic-gait abnormality with no signs of spastic paraplegia on neurological examination was classified as possibly affected.

In view of the age-dependent penetrance of ADPHSP, pairwise LOD scores for genome-screening markers were calculated by use of a conservative approach in which only affected subjects and the two oldest unaffected subjects (III:6 and III:11) were included in the analysis. Both of these unaffected subjects were clinically normal at ages 42 and 50 years, ≥ 20 years (and ≥ 3 SDs) older than the latest age at onset of disease symptoms in the family. All other family members were classified as “unknown” for purposes of the linkage analysis. After carrying out linkage analysis for 124 markers, we identified 2—D19S220 and D19S420, separated by 4.27 cM—that completely segregated with the disease. We therefore analyzed additional markers from this region. Pairwise LOD scores for these markers, derived by the conservative linkage approach described earlier, are shown in table 1. The peak pairwise LOD score was 3.208, at marker D19S420. We then repeated pairwise linkage analysis at D19S420, assigning all clinically unaffected subjects at 50% prior risk to liability classes (derived from an age-at-onset-of-symptoms/signs curve generated for the family), on the basis of the subject's age at examination. By using this analysis strategy, we generated a pairwise LOD score of 3.720 for D19S420, strongly supporting the assignment of a new ADPHSP locus (which we have termed “SPG12”) to this region. Haplotypes were constructed for markers from the region, and recombination events in affected individuals place the disease locus within a 16.1-cM interval, flanked by D19S868 and D19S902 (fig. 1). Previous cytogenetic localization of D19S220 places this locus on chromosome 19q13.1 (see The Genome Database Web site).

The age at onset of symptoms in family 28 was early, with a mean \pm SD of 6.9 ± 6.2 years. There may be locus-phenotype correlations in ADPHSP, with families showing linkage to SPG3, SPG10, and SPG12 apparently having an earlier age at onset of symptoms than is seen in families showing linkage to SPG4, SPG6, or SPG8. (Hazan et al. 1993; Hentati et al. 1994; Fink et al. 1995; Gispert et al. 1995; Dürr et al. 1996; Haug et al. 1997; Nielsen et al. 1998; Paternotte et al. 1998; Hedera et al. 1999a, 1999b; Reid et al. 1999a, 1999b, 1999c; Rocco et al. 1999). Although these apparent correlations need to be confirmed, they hint at locus-specific differences in the molecular pathology of ADPHSP.

Genes involved in formation or maintenance of the neuronal cytoskeleton or in myelination might be considered candidate genes for ADPHSP. Two such genes

have been mapped to the SPG12 region (see the GeneMap'99 Web site). The cytoskeletal-associated protein 1, glycine motif, gene (CKAP1) contains a motif that is highly conserved in certain cytoskeletal associated proteins and that may mediate association with microtubules (Watanabe et al. 1996). The myelin-associated glycoprotein gene (MAG) may be involved in the process of myelination (Barton et al. 1987). Both of these genes are positional candidates for SPG12.

In summary, the data presented in this study identify a sixth locus for ADPHSP. This is a further step in our understanding of the full extent of genetic heterogeneity of ADPHSP and will be of help in genetic counseling of families with ADPHSP. Cloning of this and other HSP genes will be vital in the understanding of the molecular pathology of these interesting conditions.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Center for Medical Genetics, Marshfield Medical Research Foundation, <http://www.marshmed.org/genetics> (for marker locations)
GeneMap '99, <http://www.ncbi.nlm.nih.gov/genemap/> (for radiation-hybrid-mapping data on candidate genes)
Genome Database, The, <http://www.gdb.org> (for cytogenetic locations of SPG12 markers)
Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for SPG3 [MIM 182600], SPG4 [MIM 182601], SPG6 [MIM 600363], SPG8 [MIM 603563], and SPG10 [MIM 604187])
UK Human Genome Mapping Project Resource Centre, <http://www.hgmp.mrc.ac.uk> (for GLUE interface and other linkage utilities)

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